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Mesophyll conductance to CO_2 is the most significant limitation to photosynthesis at different temperatures and water availabilities in Antarctic vascular species

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ABSTRACT

The impact of climate change on Antarctic plants is not only associated to the effect of increased temperature but also strongly modulated by water availability (WA) illustrating the importance of this factor in predicting responses to warming. The aim of this study was to evaluate the effect of temperature and WA on the photosynthetic performance and photosynthetic limitations of the only two Antarctic vascular species: *Deschampsia antarctica* and *Colobanthus quitensis*. We hypothesize that: the ability of Antarctic plants to increase their net CO_2 assimilation (A_N) in response to raising growth temperature would be constrained by mesophyll conductance (g_m); and decreases in water availability may counteract any benefit in carbon gain obtained upon increasing temperature. To address this issue, both species were grown (T_g) and measured (T_m) at three different temperatures (5, 10 and 16 °C). Furthermore, two different irrigation conditions (well-watered, WW, and water-deficit, WD) were applied at 16 °C. Gas-exchange measurements showed that A_N and their underlying diffusive (g_s and g_m) and biochemical components (V_{cmax}) were mainly determined by T_g and, to a lesser extent by T_m. Warmer conditions favor A_N of both species, although *D. antarctica* requires higher increases of temperature to show the same response. Changes in A_N in response to either temperature or WA are due to proportional concomitant changes of stomatal and mesophyll conductances, and carboxylation capacity. However, g_m remains the most important limitation at any environmental condition. Reduced WA can completely counteract any benefit to photosynthesis induced by raising temperature, suggesting that these species may present a quite homeostatic photosynthetic response to the climate changes predicted for the Antarctic region.

1. Introduction

Several studies have explored the possible effects of recent regional climate change on Antarctic terrestrial ecosystems (Convey, 2006, 2013; Lee et al., 2017; Sancho and Pintado, 2011). According to these studies both *Deschampsia antarctica* Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae), the only two vascular plants that naturally colonize the Antarctica, have shown increases in the size and number of their populations on several locations of the Maritime Antarctica (Cannone et al., 2016; Fowbert and Smith, 1994; Gerighausen et al., 2003). The above seem to be related to longer and warmer growing seasons, uncovered soil availability due to ice retreat and higher frequency of rains (Gerighausen et al., 2003; Grobe et al., 1997; Kappen, 1999; Smith, 1994).

Abbreviations: l_b, Biochemical limitation; C_c, chloroplast CO_2 concentration; T_g, growth temperature; V_{cmax}, maximal Rubisco carboxilation rate; T_m, measurement temperature; g_m, mesophyll conductance; l_m, mesophyll diffusion limitation; A_N, net CO_2 assimilation; PAR, photosynthetic active radiation; g_s, stomatal conductance; l_s, stomatal conductance limitation; C_i, sub-stomatal CO_2 concentration; WA, water availability; WD, water-deficit; WW, well-watered

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The two Antarctic vascular species share several morphological and physiological traits to deal with the harsh Antarctic climate: freezing tolerance, ability to maintain positive CO_2 assimilation rate near 0 °C, resistance to photoinhibitory conditions and tolerance to water stress (see Cavieres et al., 2016 and references therein). Both species have thick and tight leaf mesophyll, along with other xerophytic characteristics associated with extremely low mesophyll conductance for CO_2 (g_m) (Sáez et al., 2017). To counteract this negative effect of low g_m, and to minimize carbon losses through photorespiration, Rubisco developed remarkable high values of specificity for CO_2 in both Antarctic species (Sáez et al., 2017). Nonetheless, these two species also show important differences between them. For instance, while *D. antarctica* can tolerate freezing temperatures as low as −27 °C, deploying a series of physiological and molecular responses to freezing temperature, *C. quitensis* is more sensitive to freezing temperatures (−14 °C) and does not show similar physiological and molecular responses (see Alberdi et al., 2002; Bravo et al., 2009). Differences also occur in photoprotective mechanisms: while *C. quitensis* dissipates the excess absorbed energy through non-photochemical quenching, down regulating its electron transport rate and hence minimizing oxygen reduction and thus generation of reactive oxygen species, *D. antarctica* actively uses oxygen as an alternative electron sink through the water–water cycle (Pérez-Torres et al., 2007). Furthermore, *C. quitensis* has lower levels of antioxidant enzymes activity compared to *D. antarctica*, suggesting a low contribution of the water–water cycle to the modulation of redox state of the photosynthetic electron transport chain (Pérez-Torres et al., 2004).

Few studies have addressed the *in situ* effects of climate change on these species. In a short-term field study, Day et al. (1999) found that, while the vegetative growth of *C. quitensis* increased with warming, it decreased in *D. antarctica*. Recently Sáez et al. (2018a), using open top chambers (OTC) as a passive and continuous warming system, showed that *in situ* warming by about 3 °C increased mesophyll conductance, CO_2 assimilation and growth rate in *C. quitensis*, while almost no changes were detected in *D. antarctica*. Thus, it seems worth asking whether the contrasting responses observed in the field experiments between both species, are a consequence of the small range of temperature increases accomplished by OTCs. Other studies performed under laboratory conditions at higher temperatures (7, 12 and 20 °C) have shown positive increases of photosynthesis in *D. antarctica* as well (Xiong et al., 2000). In addition, we have observed that *D. antarctica* showed differences in chlorophyll fluorescence (mainly electron transport rate) and photosynthetic pigment concentrations when growing at 16 °C but not when growing at 10 °C compared to plants grown at 5 °C (Sáez et al., 2018b). However, all the previous reports under laboratory conditions did not address the mechanisms behind the observed photosynthetic responses, which remain to be elucidated. Increasing temperatures have been frequently reported to increase both the maximum rate of photosynthetic electron transport and the velocity of Rubisco carboxylation in many different species (Sage and Kubien, 2007). However, due to the very low mesophyll conductance (g_m) reported for the two Antarctic species (Sáez et al., 2017), perhaps any beneficial increase of raising temperature could have no effect on the observed rate of photosynthesis unless the plants are capable of simultaneously adjusting their g_m.

As a result of climate change in Antarctica, increases in the evapotranspiration rates and lower precipitation (Robinson et al., 2003) could affect the water availability for plants (Bokhorst et al., 2007; Turner et al., 2005). There is only one report analyzing the effects of water availability in Antarctic vascular plants. In this study, Day et al. (2009) studied the effects of supplemental precipitation on Antarctic plant production and abundance. They found that precipitation regime had large impacts on warming responses, illustrating the importance of future precipitation regimes in predicting responses to warming.

In the present study, we evaluated the photosynthetic performance and photosynthetic limitations of *D. antarctica* and *C. quitensis* cultivated at three thermal growing regimes. Given that water availability may limit the plant response to warming, we also evaluated the effect of water availability at the highest growing thermal regime, which coincides with the optimal temperature for photosynthesis in both Antarctic species. We hypothesize that: (i) due to the strong mesophyll conductance limitation to photosynthesis reported for both Antarctic species, their ability to increase net photosynthesis in response to increasing temperature would be constrained by the capacity of mesophyll conductance to acclimate to changes in temperature; and (ii) decreases in water availability may counteract any benefit in carbon gain obtained upon increasing temperature. To unravel the photosynthetic limitations and other morphophysiological responses in these two species may be of great value to foresee the consequences of global warming impacts on Antarctica as well as to compare with the reported for other cold ecosystems, such as alpine and arctic ecosystems.

2. Materials and methods

2.1. Plant material and growth conditions

Deschampsia antarctica and *Colobanthus quitensis* were collected from an Antarctic population located in King George Island (KGI), near to the H. Arctowski Polish Antarctic Station (62° 09′S, 58° 28′ W). The plants were transferred to the laboratory and cultivated in pots of 500 ml, in a substrate consisting of sterile organic soil, vermiculite and peat (3:1:1 v/v) in a growth chamber at 4 °C (Pi-Technology Inc. Santiago, Chile) at 80 ± 5% RH, with a light intensity of 200 μmol photons m^{-2} s^{-1} and 18 h day length. The growth chambers were specially designed for this experiment, in order to maintain the temperatures of each treatment. The light was provided by fluorescent tubes enriched with LED panels (GP-180 W, Innova-Led, Santiago, Chile), composed by 119 Bridgelux 3 W LEDs, 60° aperture angle, (60% 660 nm, 10% 630 nm, 6% 590 nm, 10% 460 nm, 4% 410 nm and 10% white). Plants were fertilized with 0.02 g L^{-1} Phostrogen (Solaris, Buckinghamshire, UK) once every two weeks. After three weeks, plants were randomly assigned to three different growth temperature regimes (T_g): 5 °C, 10 °C and 16 °C. This temperature range was chosen because they corresponded, respectively, to the highest average diurnal record of air temperature, the highest average record of leaf temperature during Antarctic summer, and the optimal photosynthetic temperature determined in *D. antarctica* and *C. quitensis* growing in KGI (Sáez et al., 2018b). To address the effects of water availability, the plants grown at 16 °C were split into two water availability treatments: field capacity (WW) and soil water deficit at 35% of field capacity (WD). The water deficit was imposed by allowing the soil to dry out until reaching the selected humidity level determined gravimetrically in each pot, which was achieved during a two months period to allow plants to progressively acclimate to the water regime. Thereafter, plants were maintained 21 days under the water condition selected before the measurements.

2.2. Leaf gas exchange and chlorophyll fluorescence

Leaf gas exchange was determined simultaneously with chlorophyll *a* fluorescence using an open gas exchange system Li-6400XT (LI-COR Inc., Lincoln, NE, USA) with an integrated fluorescence chamber head (Li-6400-40; LI-COR Inc.). The response of the net photosynthesis CO_2 uptake (A_N) to varying sub-stomatal CO_2 concentration (C_i) was studied with the so-called A_N-C_i curves. Measurements were performed in a group of leaves, trying to cover all the IRGA's chamber area but avoiding leaf overlap, according to the procedure described in Sáez et al. (2017). Conditions inside the leaf cuvette consisted of a saturating photosynthetic active radiation (PAR) of 1000 μmol photons m^{-2} s^{-1} and an air relative humidity between 40 and 50%. Three different measurement temperatures (T_m) were used for each treatment corresponding to the three growth temperatures: 5 °C, 10 °C and 16 °C. These temperatures were chosen according Sáez et al. (2018, see above). The

A_N-C_i curves were initiated by allowing the leaf to reach steady-state (typically 20–30 min after clamping the leaf) values for A_N and stomatal conductance (g_s) at 400 µmol CO_2 mol^{-1} air CO_2 concentration in the leaf chamber (C_a). Thereafter, C_a was lowered to 0 µmol CO_2 mol^{-1}, for subsequent stepwise increases up to a maximum of 2000 µmol CO_2 mol^{-1}. In total, A_N-C_i curves consisted on measurements taken after maintaining the leaf for at least 5 min at 11 different C_a. Corrections for the leakage of CO_2 into and out of the leaf chamber of the Li-6400 were applied to all gas-exchange data, as described by Flexas et al. (2007).

The fluorometer was set to multiphase pulse with factory setting, target intensity = 10 and ramp depth = 40% (Loriaux et al., 2013). The quantum efficiency of the photosystem II (PSII) was determined using the equation:

$$\phi PSII = (F'_m - F_s)/F'_m$$

where F_s is the steady-state fluorescence in the light (PPFD 1000 µmol m^{-2} s^{-1}) and F'_m the maximum fluorescence obtained by the ramp-based extrapolation after a light-saturating pulse. As φ_{PSII} represents the number of electrons transferred per photon absorbed by PSII, the electron transport rate (ETR) can be calculated as:

$$ETR = \varphi_{PSII} \cdot PPFD\ \alpha\ \beta$$

where PPFD is the photosynthetic photon flux density, α is the leaf absorptance, and β is the distribution of absorbed energy between the two photosystems, assumed to be 0.5. The leaf absorptance was directly measured using a chlorophyll fluorescence system Imaging mini-PAM (Walz, Effeltrich, Germany). This measurement requires successive illumination of the samples with red (R) and near infrared (NIR) light and the capture of each remission image. The leaf absorptance was calculated by the equipment software pixel by pixel as follows: Abs = 1- R/NIR. The leaf absorptance for *D. antarctica* during this experiment has previously been reported in Sáez et al. (2018b), and no differences were found between these values and those determined for *C. quitensis.*

2.3. Estimation of g_m and C_c

Mesophyll conductance to CO_2 (g_m) was calculated from the combined gas-exchange and chlorophyll *a* fluorescence measurements as in Harley et al. (1992):

$$g_m = A_N / (C_i - (\Gamma^* (ETR + 8 (A_N + R_L)) / (ETR - 4 (A_N + R_L))))$$

where A_N and C_i were obtained from gas exchange measurements at saturating light. The rate of non-photorespiratory CO_2 evolution in the light (R_L) was assumed to be half of dark respiration (R_{dark}), and the chloroplast CO_2 compensation point (Γ^*) was calculated according to Brooks and Farquhar (1985) from the Rubisco specificity factor ($S_{c/o}$) measured *in vitro* (Sáez et al., 2017). Determination of g_m was used to calculate C_c, converting A_N-C_i curves into A_N-C_c curves, as $C_c = C_i - (A_N/g_m)$.

The maximum carboxylation rate (V_{cmax}) was derived from A_N-C_c curves according to Farquhar et al. (1980) and using the kinetics constants for Rubisco determined for each of these two species at the three measurement temperatures (T_m) at which gas-exchange was measured, according to the values obtained from *in vitro* measurements (Sáez et al., 2017). In addition, V_{cmax} was used to calculate the active Rubisco sites as V_{cmax} / k_{cat}^c. The values of k_{cat}^c were obtained from *in vitro* measurements in Sáez et al. (2017).

2.4. Quantitative analysis of photosynthetic limitations

To separate the relative control on A_N resulting from limited stomatal conductance (l_s), mesophyll diffusion (l_m), and limited biochemical capacity (l_b) ($l_s + l_m + l_b = 1$), the quantitative limitation analysis of Jones (1985) as implemented by Grassi and Magnani (2005) was used. The limitations of the different components were calculated as:

$$l_s = ((g_{tot}/g_s)\ \delta A_N/\delta C_c)/\ ((g_{tot} + \delta A_N/\delta C_c)),$$

$$lm = ((gtot/gm)\delta AN/\delta Cc)\ /\ ((gtot + \delta AN/\delta Cc)),$$

$$lb = gtot\ /\ (gtot + \delta AN/\delta Cc),$$

where g_s is the stomatal conductance to CO_2, g_m is the mesophyll conductance according to Harley et al. (1992), and g_{tot} is the total conductance to CO_2 from ambient air to chloroplasts (sum of the inverse serial conductances g_s and g_m). $\delta A_N/\delta C_c$ was calculated as the slope of A_N-C_c response curve. At least four curves per species and growth condition were used.

2.5. Plant water status and leaf mass area

To evaluate the water status after the application of the water availability treatments, the relative water content (RWC) of leaves was evaluated. Leaves were harvested and fresh weight (FW) was immediately determined. Turgid weight (TW) was determined after placing the samples in distilled water in darkness at 4 °C to minimize respiration losses until constant weight (full turgor, typically after 24 h). Afterwards, samples were dried during 72 h at 70 °C in an oven to obtain the dry weight (DW). The relative water content (RWC) was determined as follows: RWC = (FW-DW) / (TW-DW) * 100. Six replicates per species and treatment were obtained.

The leaf mass area (LMA) was calculated as the ratio of dry mass to leaf area. Leaf area was determined in fresh leaves using pictures analyzed with image analysis software (ImageJ; Wayne Rasband/NIH, Bethesda, MD, USA). Then, the dry mass of these leaves was determined after oven drying for 72 h at 70 °C A total of six replicates were obtained per species and treatment.

2.6. Statistical analysis

A fully factorial two-way ANOVA was performed to assess differences between growth temperature (T_g: 5 °C, 10 °C and 16 °C) and measurement temperature (T_m: 5 °C, 10 °C and 16 °C) on the photosynthetic performance and their limitations. Differences among means were assessed by *a posteriori* Tukey test ($P < 0.05$). To assess the effects of water irrigation a completely randomized design and a Student *t*-test ($P < 0.05$) was performed. A Pearson correlation analysis was performed to assess the relationship between the net photosynthesis and the different components of diffusive and biochemical limitations. All these analyses were done in Statistica 7.0 (Stat Soft Inc. Tulsa OK, USA).

3. Results

3.1. Effects of temperature on photosynthesis and its underlying determinants

The net CO_2 assimilation rate (A_N) at ambient CO_2 concentration and the underlying diffusive and biochemical determinants were affected by the growth temperature (T_g) and, to a lesser extent, by the measurement temperature (T_m, Table S1). A significant interaction between these factors was found for most of the photosynthetic parameters in *D. antarctica,* but no in *C. quitensis.*

In *D. antarctica,* A_N showed no differences between plants grown at 5 °C and 10 °C, regardless on T_m, with values ranging between 1.6 ± 0.3 µmol CO_2 m^{-2} s^{-1} and 2.0 ± 0.2 µmol CO_2 m^{-2} s^{-1}. In contrast, A_N significantly increased in plants grown at 16 °C and measured at 10 and 16 °C (Fig. 1A). A similar trend was observed in the diffusive components of photosynthesis. The stomatal conductance (g_s) showed values around 0.05 mol H_2O m^{-2} s^{-1} in plants grown at 5 °C and

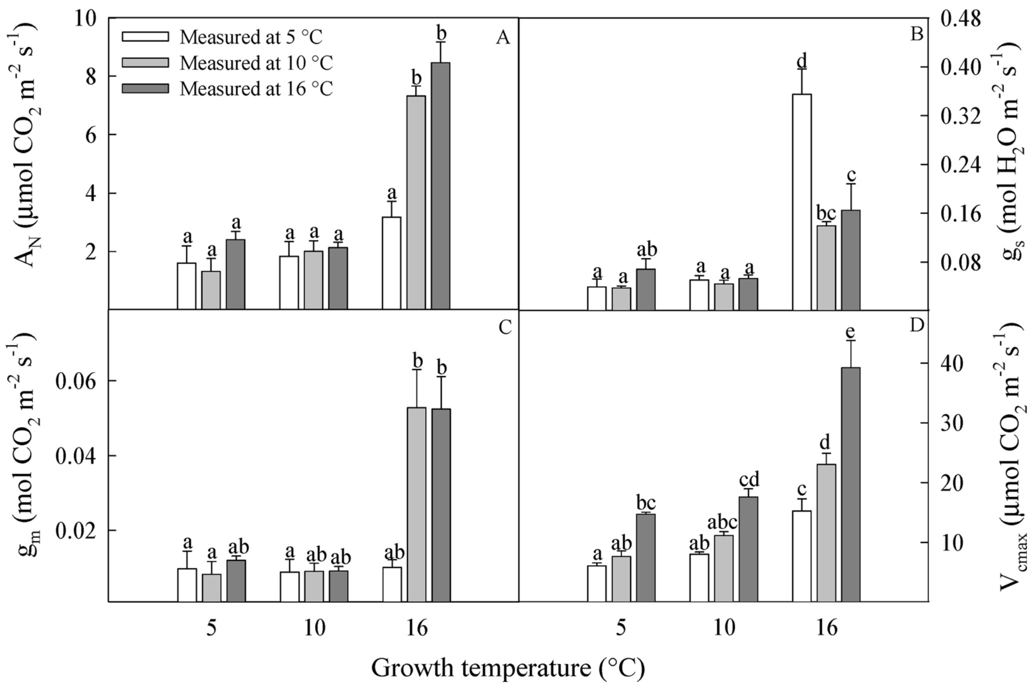

Fig. 1. The net photosynthetic CO_2 assimilation rate (A_N), the stomatal conductance (g_s), the leaf mesophyll conductance to CO_2 (g_m) and the maximum Rubisco carboxylation rate (V_{cmax}) of *D. antarctica* grown and measured at 5 °C (white bars), 10 °C (grey bars) and 16 °C (dark grey bars). Values are means ± standard error (n = 4–8). Different letters indicate statistically significant differences among growth × measurement temperatures, according to Tukey ($P < 0.05$).

10 °C, regardless on the T_m, being higher in plants grown at 16 °C (from 0.17 ± 0.04–0.35 ± 0.04 mol H_2O m^{-2} s^{-1} depending on the T_m, Fig. 1B). Likewise, the mesophyll conductance (g_m), showed values around 0.009 mol CO_2 m^{-2} s^{-1} in plants grown at 5 °C and 10 °C, significantly lower to those found in plants grown at 16 °C and measured at 10 °C (0.05 ± 0.01 mol CO_2 m^{-2} s^{-1}) and 16 °C (0.052 ± 0.009 mol CO_2 m^{-2} s^{-1}, Fig. 1C). At each T_g, the maximal Rubisco carboxylation rate (V_{cmax}) increased with increases in T_m (Fig. 1D). Thus, the lowest V_{cmax} values were determined in plants grown and measured at 5 °C (6.1 ± 0.5 µmol CO_2 m^{-2} s^{-1}), and the highest in plants grown and measured at 16 °C (39 ± 5 µmol CO_2 m^{-2} s^{-1}, Fig. 1D).

With few exceptions, a trend for a gradual increase in the photosynthetic parameters was observed in *C. quitensis*, consisting in increased values across T_m for a given T_g, and especially across T_g for a given T_m (Fig. 2). In this species, the lowest A_N values were found in plants grown at 5 °C, regardless on the T_m (around 1.8 µmol CO_2 m^{-2} s^{-1}). These values were almost two and three-fold lower to those observed in plants grown at 10 °C and 16 °C, respectively, with the highest values found in plants grown and measured at 16 °C (6.17 ± 0.63 µmol CO_2 m^{-2} s^{-1}, Fig. 2A). Lower variations were observed in g_s. This parameter showed the lowest values in plants grown and measured at 5 °C (0.072 ± 0.004 mol H_2O m^{-2} s^{-1}) with no significant differences between plants grown at 10 °C, and the highest values in plants grown at 16 °C A similar trend was observed in g_m, which deployed the lowest values in plants grown at 5 °C but measured at 10 °C (0.004 ± 0.001 mol CO_2 m^{-2} s^{-1}) and the highest in plants grown at 16 °C regardless on the T_m (around 0.02 mol CO_2 m^{-2} s^{-1}) (Fig. 2C). As in *D. antarctica,* in *C. quitensis* V_{cmax} increased significantly at each T_g

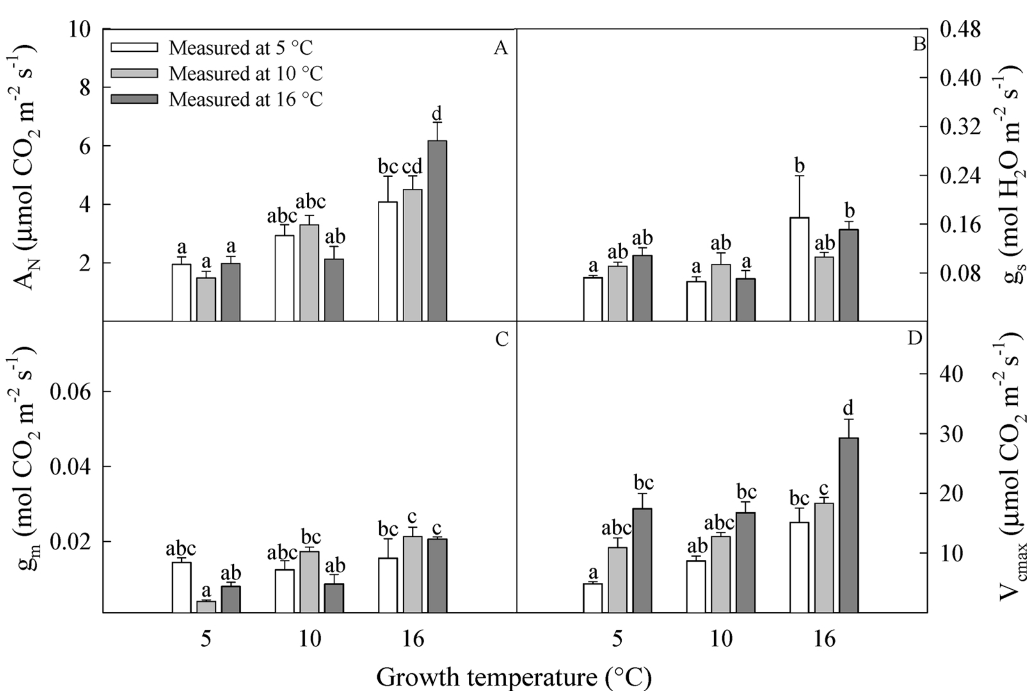

Fig. 2. The net photosynthetic CO_2 assimilation rate (A_N), the stomatal conductance (g_s), the leaf mesophyll conductance to CO_2 (g_m) and the maximum Rubisco carboxylation rate (V_{cmax}) of *C. quitensis* grown and measured at 5 °C (white bars), 10 °C (grey bars) and 16 °C (dark grey bars). Values are means ± standard error (n = 4–8). Different letters indicate statistically significant differences among growth × measurement temperatures, according to Tukey ($P < 0.05$).

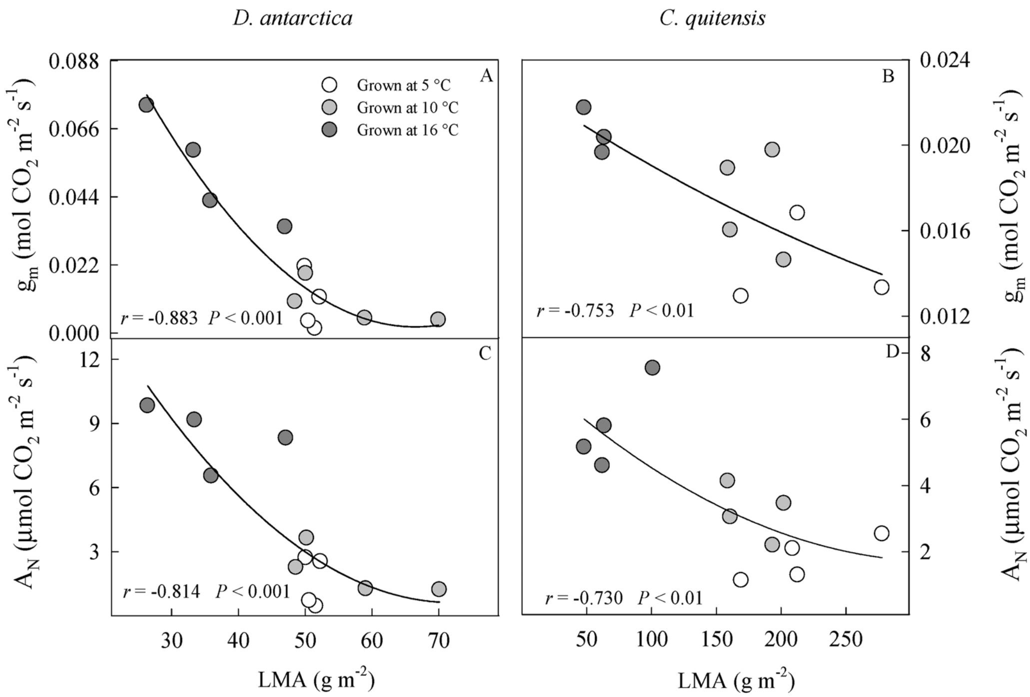

Fig. 3. Correlations of the leaf mass area (LMA) with the leaf mesophyll CO_2 conductance (g_m) and the net photosynthetic CO_2 assimilation rate (A_N) in *D. antarctica* and *C. quitensis* grown at 5 °C (white circles), 10 °C (grey circles) and 16 °C (dark grey circles). The Pearson correlation coefficients and their significances are shown considering all growing temperatures together.

with the increase in T_m. Thus, the lowest values of V_{cmax} were determined in plants grown and measured at 5 °C (4.83 ± 0.38 µmol $CO_2\,m^{-2}\,s^{-1}$), and the highest in plants grown and measured at 16 °C (29.27 ± 3.12 µmol $CO_2\,m^{-2}\,s^{-1}$, Fig. 2D).

The mesophyll conductance and the photosynthetic CO_2 assimilation rate negatively correlated with leaf mass area (LMA) in both species. Thus, the increase in g_m and A_N at higher T_g, correlated with concomitant decreases in LMA (Fig. 3). This finding indicates that the photosynthetic capacity of both Antarctic vascular species grown and measured at different temperatures is strongly determined by adjustments in the leaf structure. Indeed, when pooling all data, a strong positive correlation was found between A_N and g_{tot}, and between A_N and V_{cmax} (Fig. S1). However, this last relationship was stronger for *D. antarctica,* suggesting a co-limitation between diffusive and biochemical determinants in this species.

3.2. Quantitative analysis of photosynthetic limitations under different growth and measurement temperatures

According to quantitative limitation analysis of A_N (Fig. 4), stomatal limitations (l_s) restricted the photosynthetic capacity between 1.5 and 11.5% in both Antarctic species. Irrespective of the T_g (and also T_m), g_m was the component that mainly restricted A_N in both species, with values of mesophyll diffusion (l_m) ranging between 46–81% in *D. antarctica* and 55–78% in *C. quitensis.* In the former, l_m tended to decrease in plants grown at 16 °C and measured at 10 °C and 16 °C. In this species changes in l_m triggered changes in the biochemical capacity (l_b), with significant differences in l_b of *D. antarctica* grown at 16 °C and measured at 10 °C and 16 °C deployed l_b values close to 40%. In *C. quitensis*, l_m values were similar among the different treatments and varied between 20–40%.

3.3. Effects of water availability on the photosynthetic performance of the Antarctic plant species grown at 16 °C

The leaf relative water content (RWC) was not affected by WD in *D. antarctica* (Table 1), which showed values around 88% regardless of the irrigation treatment. In *C. quitensis*, the leaf RWC was lower in WD plants compared to WW plants. On the contrary, while an increasing tendency was observed for LMA under water stress in both species (Table 1), this was not statistically significant due to the large variability within each treatment.

Regarding the photosynthetic capacity and its underlying determinants, WD plants showed lower values compared to WW plants for both species, inducing a reduction in A_N of approximately 65% with respect to WW for both species (Table 1). The transpiration rate (E) was also significantly reduced by WD in both Antarctic species, with values around 40% lower to those found under WW conditions. The same trend was observed in g_s, g_m and V_{cmax}. As a consequence, the degree at which each limitation (l_s, l_m and l_b) restricted the CO_2 assimilation rate did not vary between water treatments in any of the species (Fig. 5). In both species and regardless of the water condition, l_m imposed the main restriction on A_N, showing values around 52% in *D. antarctica* and 68% in *C. quitensis,* followed by l_b, with values close to 38% and 26% for *D. antarctica* and *C. quitensis,* respectively.

4. Discussion

4.1. Mesophyll conductance is the most important limitation to photosynthesis in the Antarctic plant species

As previously observed in field experiments (Sáez et al., 2018a), small increases in growth temperature induced diffusional and biochemical adjustments in *C. quitensis* that resulted in higher CO_2 assimilation rates for this species but not in *D. antarctica.* In the present study, we have extended the range of growing temperatures, showing that *D. antarctica* is also able to display those adjustments in response to larger increases in growth temperature (Figs. 1 and 2). These results confirm previously reported positive effects of increases in the growth temperature for both species (Edwards and Smith, 1988; Xiong et al., 2000). However, these previous reports did not address the mechanisms behind this response, which remained to be elucidated. The present results show that, as it was observed in the field (Sáez et al., 2017, 2018a), under laboratory conditions g_m in these two Antarctic species remain markedly low. Notably, g_m responded to a larger extend to changes in the T_g than to the measurement temperature (T_m). Although g_m values from plants growing in the field were slightly higher than those determined in the present study, likely due to the moderate

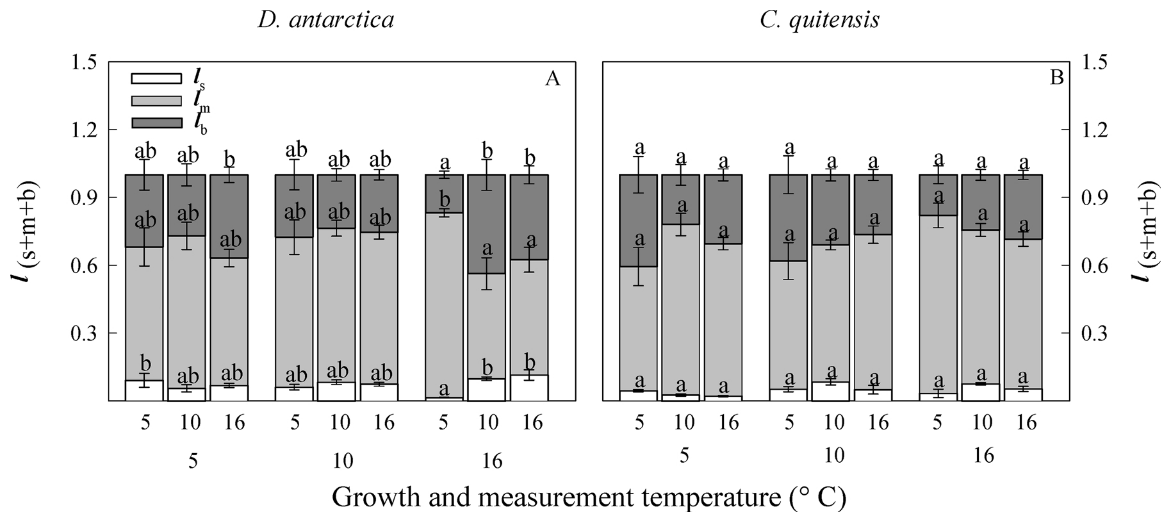

Fig. 4. Quantitative limitation analysis of the net photosynthetic CO_2 assimilation in relation to the stomatal (l_s), mesophyll (l_m), and biochemical (l_b) limitations for *D. antarctica* and *C. quitensis* grown and measured at 5 °C, 10 °C and 16 °C. Values are means ± standard error (n = 4–8). Different letters indicate statistically significant differences for each limitation among growth × measurement temperatures within each species, according to Tukey ($P < 0.05$).

growth light intensity used in the growth chambers as compared to light regimes in the field, both Antarctic species showed an increase of this diffusive component with the increase in the growth temperature. The increase in g_m, observed also in other species (i.e. Flexas et al., 2008; Niinemets et al., 2009; Peguero-Pina et al., 2012; Tomás et al., 2013), was related to decreases in LMA. It has been shown that LMA correlates negatively with variations in cell wall thickness and the surface of chloroplasts exposed to intercellular air spaces, the two anatomical traits that most limit g_m in many species (Tomás et al., 2013; Tosens et al., 2016). In addition, LMA also correlates negatively with leaf density, and hence the temperature driven increase in g_m may be also a consequence of the lower density of leaf mesophyll at higher temperature (Niinemets, 1999; Niinemets et al., 2009). Overall, the LMA effect on g_m supports the idea that changes in g_m are driven by leaf structural characteristics, as previously was observed in *C. quitensis* growing in warmer conditions in the field (Sáez et al., 2018a).

Several studies have shown that g_m tends to increase with increasing measuring temperature (Bernacchi et al., 2002; Diaz-Espejo et al., 2007; Evans and von Caemmerer, 2013; Flexas et al., 2008; Scafaro et al., 2011; Warren, 2008), likely as a result of increase in the activity of the metabolic components associated to the CO_2 diffusion (Bernacchi et al., 2002). However, stomatal conductance and the Rubisco carboxylation rate (V_{cmax}) also increase exponentially with increasing temperature (Diaz-Espejo et al., 2007; Sage and Kubien, 2007; Yamori et al., 2006). As a consequence, photosynthesis typically remains co-limited by the three limitations (stomatal, mesophyll conductance and carboxylation) at any given temperature (Flexas and Díaz-Espejo, 2015; Flexas et al., 2016). Nevertheless, in the two Antarctic species, when diffusive and biochemical determinants were quantitatively analyzed, photosynthesis was in general limited by the mesophyll component (l_m) at any

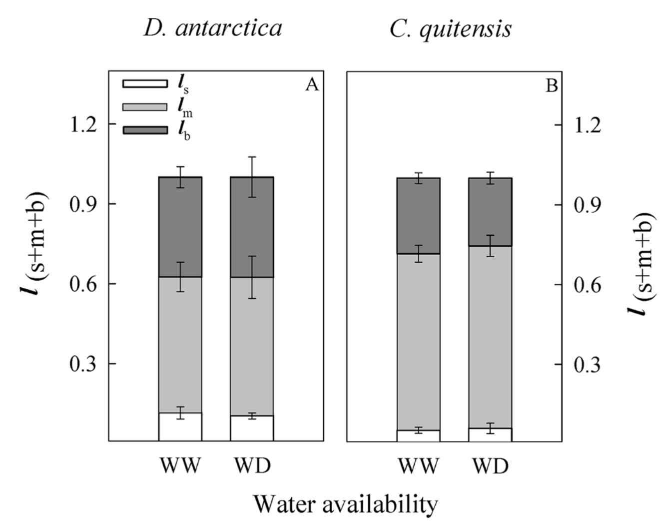

Fig. 5. Quantitative limitation analysis of photosynthetic CO_2 assimilation in relation to the stomatal (l_s), mesophyll (l_m), and biochemical (l_b) limitations of photosynthetic assimilation of *D. antarctica* and *C. quitensis* grown and measured at 16 °C under well watered (WW) and water deficit (WD) treatments. Values are means ± standard error (n = 4–6).

Table 1
The leaf relative water content (RWC), the leaf massa area (LMA), the net photosynthetic CO_2 assimilation rate (A_N), the transpiration rate (E); the stomatal conductance (g_s), the leaf mesophyll conductance to CO_2 (g_m) and the maximum Rubisco carboxylation rate (V_{cmax}) of *D. antarctica* and *C. quitensis* grown at 16 °C under well watered (WW) and water deficit (WD) treatments. Values are means ± standard error (n = 4–6). The asterisk indicates statistically significant differences between both treatments for each species, according to Student t test ($P < 0.05$).

Parameter	*D. antarctica*		*C. quitensis*	
	WW	WD	WW	WD
RWC (%)	87.90 ± 2.44	87.15 ± 3.98	80.92 ± 1.77*	70.65 ± 2.89
LMA (g m^{-2})	35.73 ± 4.29	48.45 ± 6.29	68.71 ± 11.35	84.57 ± 11.11
A_N (µmol CO_2 m^{-2} s^{-1})	8.46 ± 0.71*	2.90 ± 0.73	6.17 ± 0.63*	2.06 ± 0.39
E (mmol m^{-2} s^{-1})	1.35 ± 0.23*	0.53 ± 0.17	1.53 ± 0.31 *	0.70 ± 0.10
g_s (mol H_2O m^{-2} s^{-1})	0.165 ± 0.044*	0.055 ± 0.016	0.151 ± 0.014*	0.081 ± 0.015
g_m (mol CO_2 m^{-2} s^{-1})	0.052 ± 0.009*	0.022 ± 0.009	0.021 ± 0.001*	0.009 ± 0.002
V_{cmax} (µmol CO_2 m^{-2} s^{-1})	39.23 ± 4.57*	21.19 ± 2.03	29.27 ± 3.12*	15.05 ± 2.05

temperature (T_g or T_m, Fig. 4). The only exception to this pattern was observed in *D. antarctica* grown at 16 °C and measured at either 10 or 16 °C, when increased A_N was accompanied by a significant reduction in l_m and an increased l_b. Therefore, the mesophyll restrictions remain as the main determinant to photosynthesis in both species regardless of their growing temperature. This trait is strongly determined by the harsh Antarctic environmental conditions and the water loss control capacity. The reduction in LMA with the increase in temperature may imply changes in this capacity, and therefore make the species more susceptible to water stress (Niinemets, 2001; Wright et al., 2004).

4.2. Water deficit counteracts the positive effect of increase in growth temperature on net photosynthesis

According to Molina-Montenegro et al. (2011a, b) water stress decreases the physiological performance of Antarctic species. This is of pivotal importance in the context of climate change in Antarctica, because depending on the region or the growing season, the plants could be subject to water deficit (Convey et al., 2008; Convey, 2010; Day et al., 2009; Robinson et al., 2003).

In the present study, we evaluated the effects of water availability in plants grown at the optimal temperature (16 °C) for photosynthesis in the two Antarctic plant species, showing that water deficit (WD) resulted in a significant decrease of the leaf relative water content (RWC) only in *C. quitensis* (Table 1). This interspecific difference is likely due to stronger water conservation in *D. antarctica* exerted by the capability of its leaves to curl towards the adaxial leaf surface enclosing most of stomata and thus restricting the transpiratory surface exposed under soil water deficit (Giełwanowska et al., 2005; Romero et al., 1999). In both species, A_N was reduced by more than 65% under WD (Table 1), reaching values similar to those observed at 5 °C under WW conditions. There is evidence that water stress can reduce the photosynthetic capacity due to a decrease in the RuBP synthesis (Gimenez et al., 1992) or a decrease in Rubisco activity and/or carboxylation efficiency (Faver et al., 1996; Martin and Ruiz-Torres, 1992; Plaut and Federman, 1991). However, the reduction in the ability of the leaves to transfer CO_2 from the atmosphere to the carboxylation sites in the chloroplast stroma is the primary cause of the decline in photosynthesis under water deficit conditions (Chaves et al., 2009; Flexas et al., 2004; Grassi and Magnani, 2005). In *D. antarctica* and *C. quitensis*, a decrease in g_s, g_m and V_{cmax} was detected in response to water deficit (Table 1). The values found for all three parameters were also similar to those found in plants grown at 5 °C under WW conditions, indicating that the low temperature has the same effect that the low water availability in these species. This fact supports the idea that the traits consisting in xeric leaf anatomy, low g_m and high Rubisco specificity factor (for a review see Cavieres et al., 2016 and Sáez et al., 2017), constitute important physiological adaptations enabling these species to withstand the low temperature conditions that induce physiological drought. Concomitantly, the quantitative analysis of the photosynthetic limitations showed that, regardless of the water condition, l_m still remains the main component restringing A_N (Fig. 5), again reflecting the strong adaptive character of the traits that determine the mesophyll conductance to CO_2.

In many plant species, increases in drought leads to declines in g_s, while severe drought leads to an almost complete stomatal closure (Flexas et al., 2004; Galmés et al., 2007). The resulting reduced CO_2 availability in the chloroplasts constitutes the most important photosynthetic limitation in all these cases. In consequence, a lower light intensity is required to saturate photosynthesis under drought (Cornic, 1994; Lawlor, 1995), thus increasing the plants susceptibility to photoinhibition, especially in those environments where plants are exposed to a combination of low water availability and high solar irradiance (Galmés et al., 2007). However, this general pattern does not operate in the studied Antarctic species as g_s decreased under WD only to a similar extent as did g_m and V_{cmax} (Table 1). This is interesting, because the g_s values detected under these conditions were just above the threshold established to induce metabolic impairment in other species (Bota et al., 2004; Flexas et al., 2006, 2009), but V_{cmax} decreased to a similar extent as g_s did. It has been suggested that the decline in V_{cmax} during water stress could be due to the oxidative stress affecting Rubisco (Zhou et al., 2007). However, Antarctic plants contain very high levels of antioxidants and a great ability to manage the excess light energy (Pérez-Torres et al., 2004; 2007). Thus, it is likely that oxidative stress is not the main cause behind the observed reduction of V_{cmax}. On the other hand, it has been suggested that under WD, decreases in Rubisco activity are mainly due to decreases in the concentration of Rubisco reaction sites, either due to decreases in the enzyme concentrations or to increase in the inhibitors bound to the reaction sites (Galmés et al., 2011), although this may vary among species (Bota et al., 2004). The Antarctic plant species grow in an environment that could induce physiological drought. Moreover, due to the low temperature and strong winds, they frequently deploy low values of g_s and g_m, operating to low C_c values (Sáez et al., 2017), which could be favorable for Rubisco inactivation. Among potential adaptations to such stress conditions, interactions of Rubisco with tight-binding inhibitors would prevent Rubisco that is not being used for catalysis from being degraded by proteases (Parry et al., 2008). Therefore, under WD, deactivation of Rubisco concomitantly with a significant decrease in the number of Rubisco active sites (Fig. S2) could be associated to decreases in V_{cmax}. In many plant species, the accumulation of osmolytes such as proline, glycine betaine, and sugar alcohols under WD (Yoshiba et al., 1997) has been associated to have potential to curtail the activity of Rubisco (Sivakumar et al., 1998, 2002). The observed decrease in V_{cmax} in both Antarctic species under WD could be, also associated to an accumulation of those osmolytes. There are several reports in the literature regarding osmolyte accumulation in plants from Arctic and Antarctic habitats, mainly sugars and polyols in response to low temperature (Bascuñán-Godoy et al., 2006; Körner, 2003; Montiel and Cowan, 1993; Pastorczyk et al., 2014; Zúñiga-Feest et al., 2003) and proline in response to low temperature and salt stress (Bravo et al., 2001; Tapia-Valdebenito et al., 2016). However, there is not information on possible interactions of these osmolytes and Rubisco in these species.

A remarkable particularity of the Antarctic plant species is that, under moderate WD, they show parallel adjustments of all three photosynthetic limitations. This fact implies that, contrary to most species in which l_s increasingly results in higher water use efficiency (A_N/g_s), *C. quitensis* and *D. antarctica* may have important environmental constraints favoring the maintenance of a particular anatomy leading to strong l_m even at the expense of losing the ability to increase water use efficiency under drought. We speculate that this could be related to the ability for tolerating frequent diurnal episodes of temporary leaf dehydration due to the conditions of strong dry winds coupled with low soil water availability (mainly during episodes of frozen soil water) and high irradiance, prevalent in their natural distribution area during the growing season. Thus, when these plants are subjected to a relatively moderate water deficit, all the benefits to photosynthesis imposed by raising the growing temperature to 16 °C are fully counterbalanced, leading the plants to display A_N values similar to those of well-watered plants grown at 5 °C. Although in the context of regional climate change in Antarctica, increases in air temperature around 3 °C are predicted over the next century (Mitchell et al., 1990; Vaughan et al., 2003), at the canopy level such increases could be higher (Casanova-Katny et al., 2010) and plants may episodically be facing temperatures approaching their optimum for photosynthesis. Under these conditions, events of water deficit are expected to be more frequent and, according to our results they can fully counteract the favorable effects of increased temperature on photosynthesis.

5. Conclusions

This work constitutes the first laboratory study that quantified the photosynthetic limitations of Antarctic plant species facing increases in

the growing temperature, as well as those behind water deficit. We confirmed that warmer conditions favor the photosynthetic capacity of *C. quitensis* and *D. antarctica,* although the latter requires higher increases of temperature to show the same response. In both species, changes in A_N in response to either temperature or water deficit are due to proportional concomitant changes of stomatal and mesophyll conductances, as well as the maximum rate of Rubisco carboxylation. Because their leaf anatomy sets a very low g_m, the consequence of the proportional changes in all three limiting factors is that g_m is the most important limitation to photosynthesis in any environmental condition. This is a very particular response that differs from the patterns described for many other different species and may have some adaptive value to the particularly harsh environmental conditions in the Antarctica. On the other hand, this study shows that moderate water deficit can completely counteract any benefit to photosynthesis induced by raising temperatures, suggesting that these plants may present quite a homeostatic photosynthetic response to the climate change predicted for the Antarctic region.

Authors' contributions

PLS, LAC and LAB planned and designed the research. CFR, LP and BKR performed the measurements, collected samples and analyzed the data. PLS, JG, LAC and LAB interpreted the results. PLS drafted the manuscript with substantial contributions from JG, LAC, and LAB. MJC, and JF.

Declarations of interest

None.

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